# Oviposition Response of Green Lacewings (Neuroptera: Chrysopidae) to Aphids (Hemiptera: Aphididae) and Potential Attractants on Pecan

BRIAN A. KUNKEL[1] AND TED E. COTTRELL[2]

USDA–ARS–SAA Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008

**ABSTRACT** Pecan foliage is attacked by three species of aphids [*Monellia caryella* (Fitch), *Melanocallis caryaefoliae* (Davis), and *Monelliopsis pecanis* Bissell], resulting in damage that can reduce tree nut yield. In this study, we assayed the ovipositional response of the green lacewing *Chrysoperla rufilabris* (Burmeister) to *M. caryella* and *M. caryaefoliae* at high and low aphid densities and the development of *C. rufilabris* larvae when fed solely on each of the three pecan aphid species. During 2004 and 2005, combinations of attractants and food sprays were applied to pecan trees in an orchard to monitor green lacewing ovipositional response. We found that *C. rufilabris* laid more eggs on seedling trees infested with the *M. caryella* (at both high and low densities) than on seedlings infested with *M. caryaefoliae*. Development of *C. rufilabris* was unaffected by aphid species. At least one attractant/food spray treatment applied to trees in an orchard significantly increased green lacewing oviposition for three of the five treatment dates over both years. These results show that larvae of *C. rufilabris* will consume all aphid species attacking pecan, even though female ovipositional response can differ for aphid species. It is likely that combinations of attractants and food sprays can be used to enhance green lacewing populations in orchards.

KEY WORDS biological control, aphids, lacewings, caryophyllene, tryptophan

Three aphid species (Hemiptera: Aphididae) comprise a serious pest complex attacking pecan foliage, *Carya illinoinensis* (Wangenh.) K. Koch. The black-margined aphid, *Monellia caryella* (Fitch), black pecan aphid, *Melanocallis caryaefoliae* (Davis), and yellow pecan aphid, *Monelliopsis pecanis* Bissell damage pecan by reducing net photosynthesis, tree growth, chlorophyll content of leaves, and energy reserves (Tedders et al. 1982, Tedders and Wood 1985, Wood and Tedders 1985, 1986). Additionally, aphid feeding reduces nut volume, kernel weight, lipid content of nuts, and nut yield (Tedders and Wood 1985, Wood et al. 1985).

Aphid management in commercial pecan orchards commonly entails insecticide use (Hudson et al. 2006). Insecticide application usually is necessitated by the inability of natural enemies to regulate aphid populations in pecan orchards. Aphid populations often are poorly regulated by natural enemies that arrive late and/or disperse early (Frazer 1988). Dinkins et al. (1994) showed that neuropteran predator populations in pecan orchards lag behind pecan aphids by 1 wk. Their late arrival to an infested area may reflect prey preference or delayed detection, whereas early dispersal from an infested area may signify increasing prey scarcity. Thus, early attraction of aphidophagous insects to pecan orchards and their retention in the orchards could decrease dependence on chemical insecticides.

Artificial food sprays have been used to attract species of green lacewings (Neuroptera: Chrysopidae) into vineyards, cotton fields, and alfalfa fields (Hagen et al. 1976, White and Jubb 1980, Evans and Swallow 1993). Tassan et al. (1979) stated that food sprays incorporating both protein and carbohydrates increase the number of lacewings eggs in treated areas. Research has shown that either caryophyllene or tryptophan mixed into artificial food sprays can attract lacewings (Hagen et al. 1976, van Emden and Hagen 1976, Flint et al. 1979, McEwen et al. 1994). In addition, Dutcher (2004) found that molasses-based food sprays in pecan orchards helped reduce blackmargined aphid populations within 6–8 d. However, the potential for tryptophan or caryophyllene to attract lacewings into pecan orchards has not been studied.

Blackmargined aphids produce the most honeydew of the three pecan-feeding aphid species (Tedders 1978, Wood et al. 1987) and have been considered responsible for attracting natural enemies to pecan trees (Bumroongsook and Harris 1992, Harris and Li 1996). Nevertheless, lacewings may still oviposit rel-

atively far from food sources. Previous research suggests that honeydew-feeding adult Chrysopidae randomly oviposit without regard for aphid presence (Duelli 1984, Coderre et al. 1987). Petersen and Hunter (2002) discovered to the contrary that the green lacewings *Chrysoperla comanche* (Banks) and *Chrysopa nigricornis* Burmeister both preferred to oviposit on aphid infested pecan trees versus uninfested pecan trees. *C. comanche* adults showed a significant oviposition preference for trees infested with black pecan aphids when provided a choice of trees infested with blackmargined aphids, black pecan aphids, or no aphids; *C. nigricornis* adults showed no preference between aphid species (Petersen and Hunter 2002).

In the southeastern United States, *Chrysoperla rufilabris* (Burmeister) is a common arboreal lacewing, with adults feeding on honeydew and larvae being generalist predators of soft-bodied insects and mites (Edelson and Estes 1987). Little is known about the oviposition preference of *C. rufilabris* and the effect of each prey species of pecan aphid on development and survival. Neither *C. comanche* larvae or *C. nigricornis* larvae have shown preference for blackmargined aphids or black pecan aphids, and aphid species had no effect on survival or development time of either lacewing species (Petersen and Hunter 2001, Petersen and Hunter 2002). Previous research has shown that *C. rufilabris* consuming suboptimal food (i.e., nutritionally poor prey or under conditions of low prey availability) are unable to complete development either because of larval mortality or their inability to spin cocoons (Hydorn and Whitcomb 1979, Chen and Liu 2001). Similarly, the quantity of prey consumed by *Chrysoperla carnea* larvae affects fecundity, adult size, development time, larval weight, and food conversion efficiency (Zheng et al. 1993a, b). Thus, the impact of the different pecan aphid species on *C. rufilabris*, especially development time, is important to know because a delay in development could allow aphid populations to build and expose *C. rufilabris* larvae to intraguild predation (Tedders et al. 1990).

Our objectives were to study the ovipositional response and larval development of green lacewings to pecan aphids. Ovipositional response of *C. rufilabris* for blackmargined or black pecan aphids was examined using choice tests conducted in walk-in screen cages. Larval development was studied by rearing *C. rufilabris* on exclusive diets of blackmargined, black, or yellow pecan aphids. Additionally, field tests examined the potential of increasing oviposition by green lacewing adults using artificial food sprays and known attractants.

## Materials and Methods

**Oviposition Choice Tests.** These experiments were conducted to determine if adult *C. rufilabris* selectively chooses oviposition sites with regard to the presence of blackmargined or black pecan aphids. Pecan seedlings (25–30 cm) were grown in peat pots within a greenhouse for 8 wk before infestation with either aphid species and were used as sources of black-margined- or black pecan aphid–infested seedlings. Uninfested pecan seedlings with three to five simple leaves were placed in aphid rearing cages housing blackmargined or black pecan aphid–infested seedlings for 48 or 72 h (Cottrell et al. 2002). After infestation, test seedlings were moved outside to walk-in screen cages ($n = 16$ seedlings per cage). The cages (2.7 by 2.7 by 2.1 m) were made from 1.0-mm mesh screen supported by a polyvinylchloride (PVC) frame. Within the cage, a 1.4-m-high square frame was erected around the cage perimeter and used for placement of test seedlings within the cage. The frame consisted of four horizontal boards (244 by 10 by 5 cm) with four vertical support posts (1.4 m) at each corner. Along each of the four 2.4-m-long sides of the frame, we spaced four 473-ml plastic cups (with 31 cm between cups) and anchored them to the frame. These cups were used for locating potted pecan seedlings around the frame and allowed a convenient means of providing water to pecan seedlings.

Our first experiment compared *C. rufilabris* oviposition on aphid-infested (blackmargined or black pecan aphid) versus uninfested seedlings using only one aphid species at a time. The experiment was designed as a randomized complete block (RCBD) with each walk-in cage constituting a block. Seedling test trees were exposed to either blackmargined- or black pecan aphid–infested seedling trees for 48 h whereupon numbers of nymphs (excluding first instars) and adults per test seedling were recorded. Eight seedlings of the same treatment were arranged with four seedlings on two randomly selected adjacent sides of the stand and the eight seedlings of the other treatment being placed on the remaining two adjacent sides of the stand. We released 70 adult *C. rufilabris* (Beneficial Insectary, Redding, CA) in the center of each cage and recorded the number of eggs oviposited on each seedling after 72 h.

The second set of experiments examined the effect of aphid density on blackmargined- or black pecan aphid–infested seedlings with regard to *C. rufilabris* oviposition. Eight-week-old seedlings were exposed to blackmargined- or black pecan aphid–infested seedlings, as previously described, for 48 or 72 h to obtain test seedlings with low or high aphid densities, respectively. The arrangement of blackmargined- and black pecan aphid–infested seedlings within walk-in cages was similar to the infested versus uninfested experiment. Seedlings infested with a low density of blackmargined aphids were randomly assigned to adjacent sides of the stand and seedlings infested with a low density of black pecan aphids occupied the remaining adjacent sides. The second aphid density experiment was done the same way using seedlings infested with high densities of blackmargined or black pecan aphids. Both experiments were arranged as a randomized complete block design with each of four cages constituting a block. Seventy adult *C. rufilabris* were released into the center of each cage, and after 72 h, the number of eggs found on each seedling was recorded.

**Larval Feeding Studies.** We studied the effect of aphid species on development of *C. rufilabris* larvae. Larvae were obtained from eggs laid by *C. rufilabris* (Beneficial Insectary) and were fed blackmargined, black pecan, or yellow pecan aphids ad libitum from egg hatch to pupation. Each neonate *C. rufilabris* was placed on moistened filter paper in a 9.0-cm-diameter petri dish, and pecan leaf sections (6.0 by 4.0 cm) containing only one aphid species were added. Petri dishes were wrapped with a wax film (Parafilm, Chicago, IL) to prevent escape and maintained in an environmental chamber at $25 \pm 0.5°C$ and a 16:8 (L: D)-h photoperiod. Days from egg hatch to adult eclosion were recorded as was fresh adult weight 24 h after eclosion. No food or water was provided before weighing. This test was done two times with 15 or 19 *C. rufilabris* larvae, respectively, per aphid species. On the rare occasion that a larva was missing, it was excluded from analysis. The experiment was conducted as a completely randomized design.

**Food Sprays in Orchards.** This series of field studies examined chrysopid oviposition in an established pecan orchard. Field studies were conducted in an ≈20-yr-old mixed-variety pecan orchard located at the USDA–ARS Southeastern Fruit and Tree Nut Research Laboratory in Byron, GA. Fungicide and herbicide applications to the orchard followed conventional management practices (Hudson et al. 2006). The only insecticide application made was with imidacloprid (Provado 1.6; Bayer CropScience, Research Triangle Park, NC) using an airblast orchard sprayer at a rate of 207.0 ml product per acre on 16 July 2004. Visual observations had detected increasing aphid density, and this application was made to reduce possible confounding effects (i.e., increased prey and honeydew) from the initial aphid population before the August attractant treatments were applied. The aphid population was low in 2005.

The attractant treatments consisted of different combinations of potential food sprays (i.e., wheast [Beneficial Insectary] and 5% honey [(FMV, Inter-American Products, Cincinnati, OH)], caryophyllene, and tryptophan as follows: (1) water-sprayed control, (2) honey, (3) wheast-honey, (4) wheast-caryophyllene, (5)wheast-caryophyllene-honey, (6)wheast-tryptophan, and (7) wheast-tryptophan-honey. Our intent was to select treatment combinations that previously had not been reported on for lacewing attraction. All treatments with wheast had 1.05 kg of wheast dissolved in 3 liters of water, and all treatments with honey had a final concentration of 5% honey. Treatments with caryophyllene had 0.7 ml of (−)-*trans*-caryophyllene (Sigma Co., St. Louis, MO) applied to cotton dental wicks (Flint et al. 1979) (Darby Dental Supply, Westbury, NY). Dental wicks were attached to terminals after the corresponding wheast (treatment 4) or wheast-honey solution (treatment 5) was applied. The tryptophan treatments each had 3 g L–tryptophan (Sigma Co., St. Louis, MO) dissolved in 180 ml of 2 M HCl (Hagen et al. 1976) before being added to either the wheast (treatment 6) or wheast and honey (treatment 7) solution.

The experiment was a randomized complete block design with the orchard divided into four replicates. Two of these replicates were on opposite edges of the orchard, and two replicates were in the orchard interior, which allowed maximum spacing between replicates. Two adjacent trees were assigned to each treatment within each replicate (14 trees per replicate), and the terminals of two branches per tree were treated (i.e., four terminals per treatment per replicate) treated. Selected terminal branches were low enough to be sprayed from the ground, and the number of compound leaves per terminal ranged from 9 to 15. Adjacent trees receiving the same treatment were 12.2 m apart, whereas different treatments within replicates were separated by at least 36.6 m. Treatments were applied with a 5.7-liter hand pump sprayer (Hudson Sprayer 30151, H.D. Manufacturing, Hastings, MN). All applications were made to both the upper and lower surfaces of the leaves until treatments approached run-off.

In 2004, we applied one treatment in the spring (17 June) and two treatments during the summer (2 and 9 August). Pretreatment visual assessments were made for aphids and green lacewings within 48 h before the 17 June and 2 August application dates. Any lacewings, all stages, were removed from terminals before application of treatments. For each date, treatments were applied to the same trees but different terminals. During 2005, two summer (11 and 25 August) applications were made with a pretreatment assessment for aphids and natural enemies before the 11 August application. In both years, post-treatment assessments (96 h after application) were made for the number of green lacewings (all life stages) per terminal. Natural enemies were sampled by quickly placing a treated terminal into a white 18.75-liter container and shaking to remove adults and larvae. The terminal was visually examined for eggs, pupae, and any adults or larvae that may not have been dislodged. Numbers of green lacewings (by life stage) per terminal were recorded.

**Statistical Analysis.** Data from all experiments were square root transformed (Fry 1996) before analysis. Transformed data were analyzed using one- or two-way analysis of variance (ANOVA) (SAS Institute 2003). Mean separation was done using Tukey's honestly significant difference (HSD) when $P < 0.05$. Data are presented as nontransformed means $\pm$ SE.

## Results

**Oviposition Choice Tests.** As planned, blackmargined- and black pecan aphid–infested seedlings had significantly more aphids than uninfested seedlings where no aphids were found ($F = 101.5$; df = 1,56; $P < 0.05$ and $F = 115.4$; df = 1,56; $P < 0.05$, respectively; Fig. 1). Green lacewings discriminated between aphid-infested and uninfested seedlings in the walk-in cages by laying significantly more eggs on seedlings infested with blackmargined aphids ($F = 35.5$; df = 1,56; $P < 0.05$; Fig. 1) or black pecan aphids ($F = 31.5$;
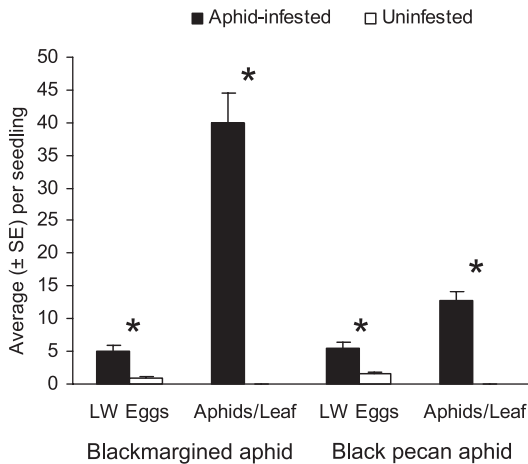
**Fig. 1.** Oviposition by *C. rufilabris* and aphid density on pecan seedlings with regard to aphid presence (infested or uninfested) and aphid species (blackmargined or black pecan aphid). *Significant difference between paired columns (*P* < 0.05). LW, lacewing.



**Fig. 2.** Oviposition by *C. rufilabris* and aphid density on pecan seedlings with regard to aphid abundance (low or high density) and aphid species (blackmargined or black pecan aphid). *Significant difference between paired columns (*P* < 0.05). LW, lacewing.

df = 1,56; *P* < 0.05; Fig. 1) compared with uninfested seedlings.

The seedlings infested with black pecan aphids had significantly more aphids per leaf than seedlings infested with blackmargined aphid in both the low aphid density trial (*F* = 11.1; df = 1,56; *P* < 0.05; Fig. 2) and the high density aphid trial (*F* = 6.4; df = 1,56; *P* < 0.05; Fig. 2). Nonetheless, *C. rufilabris* adults laid significantly more eggs on seedlings infested with blackmargined aphids than black pecan aphids in both the low and high aphid density trials (*F* = 5.3; df = 1,56; *P* < 0.05 and *F* = 5.7; df = 1,56; *P* < 0.05, respectively; Fig. 2).

**Larval Feeding Studies.** Combining the two larval feeding trials did not result in a significant interaction by trial for days to adult eclosion (*F* = 2.13; df = 2,56; *P* > 0.05) nor adult fresh weight (*F* = 0.48; df = 3,55; *P* > 0.05). The mean (±SE) days from egg hatch to adult eclosion for lacewings consuming blackmargined, black pecan, or yellow pecan aphids was 18.23 ± 0.13, 18.43 ± 0.23, and 18.80 ± 0.22 d, respectively, and was not significantly different (*F* = 2.48; df = 2,58; *P* > 0.05). Adult freshweights for *C. rufilabris* fed blackmargined, black pecan, or yellow pecan aphids were 8.12 ± 0.34, 7.51 ± 0.38, and 7.16 ± 0.24 mg, respectively, and were not significantly different (*F* = 2.40; df = 2,58; *P* > 0.05). Although *C. rufilabris* larvae suffered 19.7 ± 9.7, 59.6 ± 33.3, or 12.9 ± 2.9% mortality when they consumed blackmargined, black pecan, or yellow pecan aphids, respectively, no significant difference in mortality was detected (*F* = 1.2; df = 2,2; *P* > 0.05).

**Food Sprays in Orchards.** Pretreatment pecan aphid sampling (all species combined) for the 2004 spring (17 June) and first summer (2 August) applications revealed a mean of 1.22 ± 0.14 and 2.47 ± 0.43 (SE) aphids per compound leaf, respectively. Pretreatment chrysopid counts (all life stages) averaged 1.35 ± 0.17
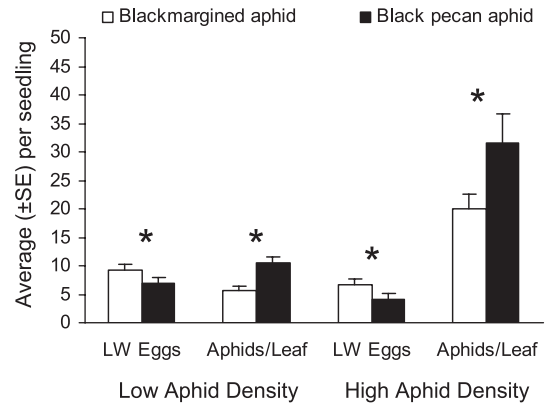
and 0.08 ± 0.03 per terminal for spring and summer applications, respectively. In these pretreatment counts, the egg stage comprised 87 and 22% of sampled green lacewings during the spring and summer, respectively. The spring application of attractants had a significant treatment effect but only for the number of lacewing eggs on wheast-tryptophan versus honey-treated terminals (*F* = 2.50; df = 6,84; *P* < 0.05; Fig. 3). No treatments were different from the control. The 2 August application had a significant treatment effect (*F* = 2.95, df = 6,84, *P* < 0.05) but only for oviposition on terminals treated with wheast-honey versus the control (Fig. 3). For the application on 9 August, there was a significant treatment effect (*F* = 3.13, df = 6,84, *P* < 0.05), but no treatment had significantly more eggs than control terminals (Fig. 3). In this trial, wheast-caryophyllene-honey had more eggs than wheast-honey and wheast-caryophyllene. Rainfall probably impacted this application because precipitation during the 96-h interval from application to evaluation was 5.7 cm but 0.0 and 1.4 cm for the 17 June and 2 August applications, respectively.

In 2005, pretreatment pecan aphid densities (all species combined) before the first summer application (11 August) were 0.84 ± 0.09 aphids per compound leaf. Pretreatment green lacewing sampling revealed 0.30 ± 0.06 per terminal, of which 32.4% were in the egg stage. The 11 August application significantly increased the number of lacewing eggs per terminal for two treatments (*F* = 3.90; df = 6,84; *P* < 0.05; Fig. 4.). Significantly more chrysopid eggs were laid on terminals treated with wheast-honey and wheast-caryophyllene than control terminals. The 25 August application revealed a significant treatment effect (*F* = 4.24; df = 6,84; *P* < 0.05; Fig. 3), with oviposition on terminals treated with wheast-caryophyllene-honey and wheast-tryptophan-honey being significantly greater than oviposition on control terminals.
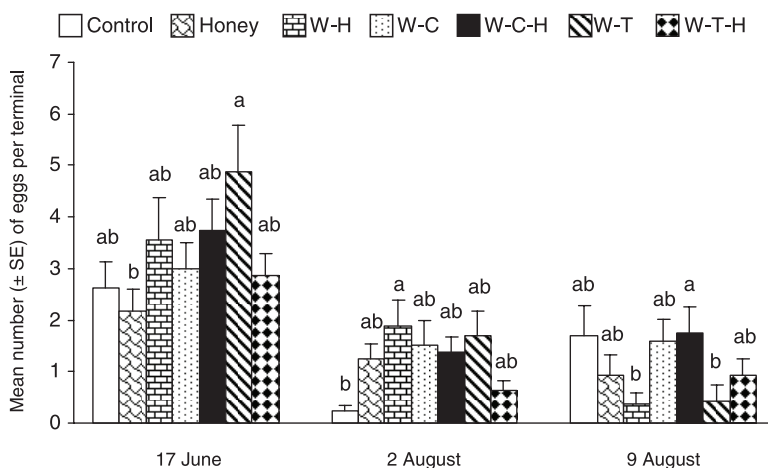
**Fig. 3.** Oviposition by green lacewings (Neuroptera: Chrysopidae) on pecan terminals in an orchard when attractants were applied on three dates in 2004. Within each date, unlike letters above columns indicates significant difference ($P < 0.05$). W-H, wheast-honey; W-C, wheast-caryophyllene; W-C-H, wheast-caryophyllene-honey; W-T, wheast-tryptophan; W-T-H, wheast-tryptophan-honey.

## Discussion

We found that *C. rufilabris* laid more eggs on aphid-infested rather than uninfested pecan seedlings, thus showing that egg placement was not random; some species have been documented to lay eggs in locations without aphid colonies (Duelli 1984, Coderre et al. 1987, Duelli 1987). In oviposition choice tests between aphid species, black pecan aphid numbers were always higher than blackmargined aphid numbers in both the low- and high-density trials. Even with this discrepancy, we found that *C. rufilabris* always laid more eggs on seedlings infested with blackmargined aphids. Blackmargined aphids produce more honeydew than either black or yellow pecan aphids (Wood et al. 1987), and the honeydew produced by black-

margined aphids may be responsible for initial attraction of natural enemies to pecan trees (Harris and Li 1996). In addition, Liao et al. (1984) found that *C. rufilabris* oviposition increased as blackmargined aphid density increased in pecan orchards. Although the green lacewing *C. comanche* also selectively chooses aphid-infested seedling trees for oviposition, this species laid more eggs on seedlings infested with the black pecan aphid than on trees infested with the blackmargined aphid (Petersen and Hunter 2002).

Even though initial attraction of adult lacewings to aphid infestations may be influenced by aphid species, we found no difference caused by aphid species when *C. rufilabris* larvae were reared solely on each of the species. Hydorn and Whitcomb (1979) and Petersen
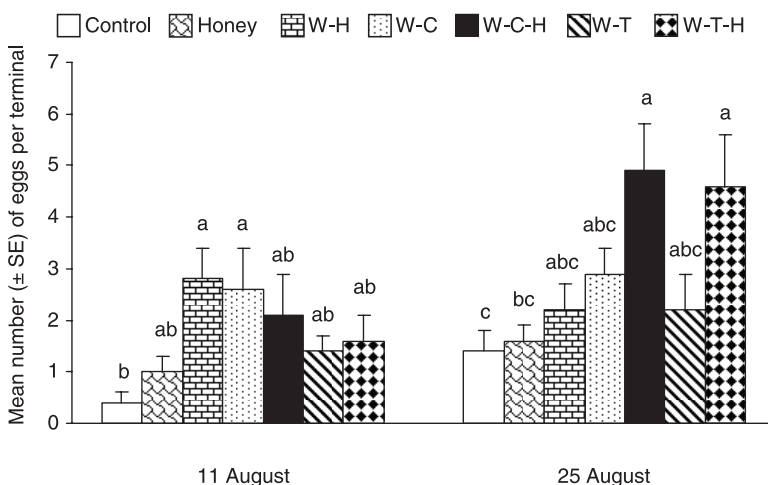


**Fig. 4.** Oviposition by green lacewings (Neuroptera: Chrysopidae) on pecan terminals in an orchard when attractants were applied on two dates in 2005. Within each date, unlike letters above columns indicates significant difference ($P < 0.05$). W-H, wheast-honey; W-C, wheast-caryophyllene; W-C-H, wheast-caryophyllene-honey; W-T, wheast-tryptophan; W-T-H, wheast-tryptophan-honey.

and Hunter (2002) reported similar results for rearing *C. rufilabris* on mixed aphid species and rearing *C. comanche* and *C. nigricornis* on pecan aphids, respectively. Thus, it is likely that larval acceptance of the different pecan aphid species in mixed populations will be similar on initial encounter, even though female response to aphid species is different.

High protein food sprays can attract and increase oviposition in treated areas within 24 h; however, effects may be short lived and influenced by quality and quantity of natural food availability (Tassan et al. 1979, McEwen et al. 1994). This was our reasoning for managing the increasing aphid population in our study orchard during 2004. As such, the pretreatment aphid populations during 2004, and also in 2005, were low.

Tryptophan applications have been shown to attract *C. carnea* to olive trees and field crops (Hagen et al. 1976, Tassan et al. 1979, McEwen et al. 1994), and caryophyllene can attract *C. carnea* to cotton fields (Flint et al. 1979). For two of the four summer applications, over both years, at least one treatment with tryptophan or caryophyllene significantly increased lacewing oviposition on pecan compared with water-sprayed control terminals. Although tryptophan is not volatile (van Emden and Hagen 1976), previous research suggests its attractive properties probably result from its breakdown through oxidative reactions. Interestingly, the presence of β-caryophyllene has been found attractive to both predaceous mites and entomopathogenic nematodes (Kong et al. 2005, Rasmann et al. 2005).

The attraction of natural enemies and their retention in pecan orchards should enhance biological control efforts against pecan aphids. Artificial honeydew has been used to increase lacewing oviposition in potatoes, vineyards, and alfalfa (Ben Saad and Bishop 1976, White and Jubb 1980, Evans and Swallow 1993). On pecan, Dutcher (2004) applied a molasses-based spray to trees that had low blackmargined aphid populations and found increased ladybeetle and lacewing populations within 6–8 d after application. Although ground covers, food sprays, and fire ant management have been studied previously (Bugg and Dutcher 1993, Smith et al. 1996, Dutcher et al. 1999, Dutcher 2004), the use of artificial attractants in combination with food sprays in pecan orchards had not been evaluated. For three of five field applications, lacewing egg counts were higher for at least one treatment compared with controls. Results from the 9 August 2004 application did not follow general oviposition trends of other application dates most likely because of deteriorating weather conditions from Tropical Storm Bonnie passing through the area soon after application. Liao et al. (1985) reported that *C. rufilabris* larvae released into closed cages at a rate of one larva per 10 leaves (when pecan aphid populations were increasing) was sufficient to prevent aphid outbreaks. The use of attractants with food sprays in pecan orchards shows potential, and their use combined with other natural enemy conservation tactics warrants further study.

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